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Research

Diversity and evolution of ectomycorrhizal host associations in the Sclerodermatineae (Boletales, Basidiomycota)

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Summary

- This study uses phylogenetic analysis of the Sclerodermatineae to reconstruct the evolution of ectomycorrhizal host associations in the group using divergence dating, ancestral range and ancestral state reconstructions.
- Supermatrix and supertree analysis were used to create the most inclusive phylogeny for the Sclerodermatineae. Divergence dates were estimated in BEAST. Lagrange was used to reconstruct ancestral ranges. BayesTraits was used to reconstruct ectomycorrhizal host associations using extant host associations with data derived from literature sources.
- The supermatrix data set was combined with internal transcribed spacer (ITS) data sets for *Astraeus*, *Calostoma*, and *Pisolithus* to produce a 168 operational taxonomic unit (OTU) supertree. The ensuing analysis estimated that basal Sclerodermatineae originated in the late Cretaceous while major genera diversified near the mid Cenozoic. Asia and North America are the most probable ancestral areas for all Sclerodermatineae, and angiosperms, primarily rosids, are the most probable ancestral hosts.
- Evolution in the Sclerodermatineae follows the biogeographic history of disjunct plant communities associated with early Cenozoic mesophytic forests and a boreotropical history. Broad geographic distributions are observed in the most promiscuous Sclerodermatineae (those with broad host ranges), while those with relatively limited distribution have fewer documented ectomycorrhizal associations. This suggests that ectomycorrhizal generalists have greater dispersal capabilities than specialists.

Introduction

The Sclerodermatineae is a monophyletic assemblage of hymenomycetes (mushroom-forming fungi) and gasteromycetes ('puff ball'-forming fungi) in the Boletales (Basidiomycota). The group presently includes 78 described species in nine genera, including six gasteromycete genera (*Astraeus*, *Calostoma*, *Diplocystis*, *Pisolithus*, *Scleroderma* and *Tremellogaster*) and three hymenomycete genera (*Boletinellus*, *Gyroporus* and *Phlebopus*)(Kirk *et al.*, 2008). Since its description by Binder & Bresinsky (2002) there have been several phylogenetic studies that involve the Sclerodermatineae (Binder & Hibbett, 2006; Louzan *et al.*, 2007; Wilson *et al.*, 2011). Of these, Louzan *et al.* (2007) had the greatest taxonomic sampling, using 43 sequences of nuclear ribosomal large subunit (25S), representing 32 species. Binder & Hibbett (2006) carried out the most character-rich study, using five genes (16S, 25S, 5.8S, mitochondrial ATPase subunit 6 (atp6) and mitochondrial large subunit (mtLSU)), but from only seven species. Recent studies describe numerous cryptic species within the Sclerodermatineae and have contributed to the taxonomic expansion of the group (Martín *et al.*, 2002; Læssøe & Jalink, 2004; Phosri *et al.*, 2007; Binder *et al.*, 2009). Here, we present a comprehensive phylogenetic analysis of the Sclerodermatineae, using an inclusive sampling of taxa and molecular sequences, to evaluate taxonomic relationships and examine patterns of age, ancestral ranges, and ectomycorrhizal host associations within the suborder.

Most Sclerodermatineae are considered to be ectomycorrhizal (Binder & Hibbett, 2006; Wilson *et al.*, 2007). For example, *Pisolithus* and *Scleroderma* are used in reforestation projects because they can form ectomycorrhizas with multiple species of host trees (Molina & Trappe, 1982b; Danielson, 1984; Sanon *et al.*, 2009). However, the ectomycorrhizal roles of *Phlebopus* and *Boletinellus* are suspect. These genera constitute the Boletinellaceae, which is an early-diverging lineage within the Sclerodermatineae. *Boletinellus* has been described as the 'ash bolete', but its association with *Fraxinus* spp. is poorly understood and may be a tripartite relationship involving an arthropod (aphid) intermediate (Tedersoo *et al.*, 2009). The ecology of *Phlebopus* is ambiguous; some studies have reported that species of *Phlebopus* can be cultivated as saprotrophs (Thoen & Ducousso, 1989; Ji

et al., 2010), while others suggest they are ectomycorrhizal fungi (Sanmee *et al.*, 2010). Species of *Phlebopus* produce large fruiting bodies that are collected from a wide range of habitats, including grasslands in Africa and Australia (which lack ectomycorrhizal hosts), fragmented forests in Argentina and Bolivia, and Southeast Asian forests of Dipterocarpaceae.

Members of the Sclerodermatineae have been reported to form partnerships with diverse hosts. However, the methods used to identify ectomycorrhizal hosts vary widely among studies. *Astraeus*, *Calostoma*, *Pisolithus* and *Scleroderma* have been conclusively shown to form ectomycorrhizas with angiosperms and gymnosperms through synthesis studies (Molina, 1981; Molina & Trappe, 1982a; Danielson, 1984) and molecular analyses (Tedersoo *et al.*, 2007; Wilson *et al.*, 2007). Unfortunately, there are many cases where the determination of ectomycorrhizal host is based on the observed proximity of fungus and putative hosts. For example, the designation of *Gyroporus* as ectomycorrhizal with oak (*Quercus*) and pine (*Pinus*) appears to be based solely on field observations (Agerer, 1999; Raidl *et al.* 2006).

To reconstruct the evolution of ectomycorrhizal associations, several factors must be considered, including the relative ages and ancestral geographic ranges of the fungi and their plant hosts. The relative ages of Boletales and their prospective hosts were addressed by Hibbett & Matheny (2009), who suggested that the Boletales are younger than angiosperms and conifers, but slightly older than the rosids, which contain many ectomycorrhizal partners of extant Sclerodermatineae. The oldest fossil attributed to Boletales is an Eocene ectomycorrhiza on *Pinus* that was interpreted as a member of the Suillineae (LePage *et al.*, 1997). The current range of the Sclerodermatineae is broad, with some genera (*Pisolithus* and *Scleroderma*) occurring on all major continents except Antarctica, while others have ranges limited to a few continents (*Calostoma*), or small geographic areas (*Diplocystis* and *Tremellogaster*). The only phylogeographic study in Sclerodermatineae to date is that of Martín *et al.* (2002), who studied distributions of *Pisolithus* and its hosts.

This study had four main goals: (1) to assemble a maximally inclusive phylogenetic tree for the Sclerodermatineae by combining trees from internal transcribed spacer (ITS) data sets with a multi-gene supermatrix data set using supertree analyses; (2) to compile host association data from the literature for Sclerodermatineae taxa, and classify the different methods used to determine host associations; (3) to use molecular dating analysis to estimate the ages of Sclerodermatineae groups and reconstruct their ancestral geographic ranges; and (4) to reconstruct the evolution of host associations in the Sclerodermatineae.

Materials and Methods

DNA extraction, PCR, and cycle sequencing

The molecular techniques in this study, including primers and the method for cloning PCR product, that were used to obtain nucleotide sequence data from the sporocarps of Sclerodermatineae taxa are described in Wilson *et al.* (2011).

Data sets

We aligned sequences using CLUSTAL X 1.81 (Thompson *et al.*, 1997) with default settings, followed by manual alignment using MACCLADE v 4.03 (Maddison & Maddison, 2005). Taxa and sequence information for all data used in this analysis are listed in Supporting Information Notes S1.

The Sclerodermatineae supermatrix data set comprises 112 operational taxonomic units (OTUs) (106 ingroup OTUs) represented by nuclear ribosomal and protein-coding genes. We generated 217 sequences (68 25S, 41 ITS, 37 RNA polymerase II subunit 1 (RPB1) and 40 subunit 2 (RPB2) genes, and 31 translation elongation factor 1a (ef1a)) and acquired 41 sequences from GenBank (44 25S, seven ITS, seven RPB1, eight RPB2, and eight ef1α). Only 5.8S rRNA data from ITS sequences were used in the supermatrix because of the high level of sequence variability in the ITS1 and ITS2 regions. Both nuclear ribosomal DNA and protein-coding sequences are present in 49 OTUs. The remaining 63 OTUs are represented by 25S rRNA sequence data only. A total of 687 characters were removed from the 4947-character supermatrix because of the variability of intron regions.

We assembled three separate ITS data sets for *Astraeus*, *Calostoma* and *Pisolithus*. The *Astraeus* data set consists of 22 ITS sequences, of which we generated five; the rest were obtained from Phosri *et al.* (2007). The *Pisolithus* data set consists of 37 sequences. We produced six of these sequences and obtained the rest from Martín *et al.* (2002). The *Calostoma* data set consists of 24 ITS sequences that we generated (except *Calostoma* sp. EU543222). The ITS phylogenies generated for supertree analyses were rooted with the most basal taxon for the genus, as indicated in the supermatrix analyses. *Gyroporus castanaeus* (EU718099) and *Gyroporus cyanescens* (EU718102) were added to each of the ITS data sets to serve as the outgroup for the Bayesian phylogenies presented in Notes S3. Sequences in each data set were aligned across the entire ITS region (ITS1, 5.8S and ITS2). The following figures represent the numbers of characters excluded from phylogenetic analyses because of ambiguities in character alignment, over the number of characters in the ITS alignments: *Astraeus* = 99/843, *Calostoma* = 310/912, *Pisolithus* = 123/793.

Phylogenetic analysis

Phylogenetic analyses were performed on a Macintosh G5 and a Linux cluster in the Clark University Center for Scientific Computing. Bayesian and maximum likelihood methods were used to produce phylogenies from the Sclerodermatineae supermatrix and all three ITS data sets. The phylogenies from these data sets were incorporated into a supertree analysis using the technique of matrix representation using parsimony (MRP), which produced the supertree data matrix that was analyzed using parsimony.

Bayesian Metropolis-coupled Markov-chain Monte Carlo analyses were performed using the GTR + I + G model of evolution in MRBAYES v. 3.1.2 (Ronquist & Huelsenbeck, 2003). The analyses used four chains, sampling every 100th tree for

10 million generations. The burn-in value for each analysis was determined by charting likelihoods of trees and removing those before the chains converged around a stable average likelihood. We reported posterior probabilities ≥ 0.95 and considered probabilities ≥ 0.98 to constitute strong support for clades.

Maximum likelihood tree generation and bootstrap analyses were performed using RAxML v. 2.2.3 (Stamatakis, 2006). Thousand bootstrap replicates were performed under the GTR + I + G model of evolution. Bootstrap support ≥ 80% was reported on branches, with ≥ 90% considered to be strong support.

For supertree analyses, the MRP data matrix was generated by Clann (Creevey & McInerney, 2005). The data matrix from the four combined Bayesian phylogenies contained 163 characters representing 168 OTUs (112 OTUs from the Sclerodermatineae supermatrix, 18 new OTUs from *Astraeus*, five from *Calostoma*, and 33 from *Pisolithus*).

We analyzed the MRP matrix using parsimony implemented in paup* v. 4.0 beta 10 (Swofford, 2003). The parsimony analysis used heuristic searches with 1000 random addition sequence replicates with TBR branch swapping and keeping 10 trees per replicate. In the initial stages of producing the supertree, some of the taxa 'misbehaved' in the sense that taxa from one group would migrate into an unrelated group (e.g. a *Calostoma* taxon would wind up in the *Gyroporus* clade). To control this 'misbehaving', a backbone constraint was enforced to maintain the integrity of known clades in supertree production. MRP characters were given a weight of 0.5–1 corresponding to RAxML likelihood bootstrap scores of 50–100% from the supermatrix and ITS phylogenies. Characters with ≤ 50% bootstrap support were given a score of 0.5. Parsimony bootstrap analyses were performed on the MRP matrix with 1000 replicates using heuristic search methods with random taxonomic addition analyses, character states sampled in proportion to their weights per bootstrap replicate, SPR branch swapping, and saving 10 trees per replicate.

Divergence time estimation

To estimate the ages of divergence events in the Sclerodermatineae, we used the secondary calibration procedure described by Renner (2005) and employed by Matheny *et al.* (2009), Skrede *et al.* (2011), and Ryberg & Matheny (2011) using BEAST v.1.6.1 (Drummond *et al.*, 2006; Drummond & Rambaut, 2007). We used BEAUTi v.1.6.1 to create XML files with the following analytical settings: GTR model, uncorrelated relaxed clock with lognormal rate distribution; estimating separate rates for genes 5.8S, 25S, RPB1, RPB2 and ef1α while using two codon partitions ((1 + 2), 3) for RPB1, RPB2 and ef1α; Tree Prior set to Yule Process speciation; running 10 million generations, sampling every 1000th tree. Each analysis was run three times. The first 10% of the trees were removed as the burn-in and the remaining trees were combined using LogCombiner v1.6.1. A summary tree was produced using Tree Annotator v1.6.1 (Drummond & Rambaut, 2007). Means and 95% highest posterior densities (HPDs) for nodes of interest were examined from BEAST logfiles using Tracer v1.5 (Drummond & Rambaut, 2007).

Part 1 of the BEAST analysis used an 18-taxon data set (Notes S7). Taxonomic groups to estimate time to most recent common ancestor (tMRCA) were defined in BEAUTi. These include the Agaricales, Boletales, Boletineae, Coniophoineae, Core Sclerodermatineae, Sclerodermatineae, Suillineae, marasmioid fungi and Tapinellineae. Two nodes were calibrated using fossil data. The marasmioid fungi (*Marasmius rotula* and *Mycena amabillisima*) were calibrated based on a 90-Ma fossil *Archaeomarasmius legetti* from mid-Cretaceous amber (Hibbett *et al.*, 1997). In BEAUTi this was set as an exponential prior with an offset of 90 and mean of 10. The Suillineae (*Suillus pictus* and *Gomphus roseus*) were calibrated using a 50-Ma permineralized suilloid ectomycorrhiza fossil associated with Pinaceae roots (LePage *et al.*, 1997). In BEAUTi this prior was set as an exponential distribution with an offset of 50 and a mean of 25. This mean was used to incorporate a 140-Ma date, the age of the oldest known fossil in the Pinaceae (LePage, 2003), within the 95% HPD. This prior sets up a likely age range for the Pinaceae ectomycorrhizal association and probable age of the Suillineae.

Part 2 of the BEAST analysis used a 58-taxon data set (a subset of the 112-taxon supermatrix data set) with taxa selected for molecular dating and phylogeographic analysis (Notes S1). Groups used to evaluate tMRCAs include: Suillineae, Sclerodermatineae, Core Sclerodermatineae, *Astraeus*, Bolletinellaceae (*Boletus* and *Phlebopus*), *Calostoma*, Diplocystidiaceae, *Gyroporus, Pisolithus* and *Scleroderma*. One node, the Suillineae, was calibrated with Pinaceae fossils using the priors described in the preceding paragraph. Two nodes, the Sclerodermatineae and Core Sclerodermatineae, were calibrated using a lognormal distribution with offset, mean and stdev set to approximate the age and HPD of these nodes as estimated in the first part of this analysis.

Ancestral range reconstruction

To reconstruct the ancestral ranges for major groups of Sclerodermatineae, we used dispersal-extinction-cladogenesis (DEC) analysis developed by Ree & Smith (2008). These analyses were performed using the consensus phylogeny produced in the divergence time analysis. Scripts for analysis were produced using the Lagrange configurator (www.reelab.net/lagrange). Definitions of areas, range constraints and the dispersal rates for models are presented in Fig. 4(e). Seven areas were defined: North America, Central America, Asia, Southeast Asia, Europe, Africa and Australasia. Each taxon in our data set was assigned to one area based on the origin of the collection representing that taxon (Fig. 3). We tested the effect of range limitations on Sclerodermatineae ancestors by constraining ancestral ranges to either two or three areas (Fig. 4e).

To evaluate variation in dispersal rates, area matrices were assembled under two model criteria. First, the restricted dispersal model allows for a dispersal rate of 1.0 between adjacent areas, but nonadjacent areas are given a dispersal rate of 0. Secondly, the relaxed dispersal model uses the same rate of dispersal between adjacent areas, but allows for dispersal between non-adjacent areas using a reduced rate of 0.5 and 0.25 for

species dispersal to two and three areas, respectively, away from the original range (see Fig. 3 and 4e). Because land masses have changed over geological history, organismal rates of dispersal between continents are affected by the availability of land bridges and migration routes that existed at different times. For both models, the area matrices shown in Fig. 3 define rates of dispersal for different geological time frames used in this analysis. These are divided among five time frames which are displayed in Fig. 3 and defined in Table 1.

Extant host associations in *Calostoma*

Host associations in *Calostoma sarasini* and *Calostoma retisporum* were studied in the Malaysian Provinces of Selangor, Negeri Sembilan and Sabah in January 2006, and May and December 2007. Fruiting bodies were collected and dried with a portion of the fruiting bodies stored in 1× CTAB or silica gel for future DNA extraction. Soil cores from directly beneath the fruiting bodies were extracted and sifted for ectomycorrhizal root tips, which were stored individually in 1× CTAB buffer. Fungal and plant DNA were isolated from ectomycorrhizal roots. PCR and cycle sequencing of fungal DNA from ectomycorrhizal root tips followed protocols described in Wilson *et al.* (2007), while analyses of plant DNA used primers rbcL-F1 and rbcL-R1 and protocols described in Sato *et al.* (2007) to amplify the ribulose biphosphate carboxylaze chloroplast gene (rbcL). Fungal ITS and plant rbcL sequences were used as queries in BLAST searches of GenBank.

Extant host associations in the Sclerodermatineae

We performed a literature search to survey the documented symbiotic partners for Sclerodermatineae taxa. We classified the data source as either (A) field collections/observations, or (B) data generated from laboratory/synthesis experiments. Four categories were created to classify the method used to identify the taxa involved in ectomycorrhizal associations: (1) molecular analysis, (2) rhizomorph tracing from fruit body to ectomycorrhizas, (3) morphological identification of ectomycorrhizas, and (4) association of fruiting bodies with nearby hosts.

We used the classifications described above to define host identifications as either stringent or liberal. Stringent definitions of host association use methods that demonstrate physical or molecular evidence for an association, including molecular analyses, synthesis studies, or, in some rare cases, tracing of fruiting body rhizomorphs to root tips occurring in mono-dominant stands. The remaining host determination methods were defined as liberal host identifications. Unpublished observations of host association are listed as pers. comm. or 'this study'. All host association data described here are given in Notes S2.

Table 1 Geological events used to define age ranges for dispersal-extinction-cladogenesis (DEC) analysis priors

DEC prior (Ma)	Event	Event marker in Fig. 3
90–50	Global land-masses leading to the break-up of Laurasia (North America–Europe) and Gondwana (Australia–South America via Antarctica)	A, B
50–20	After the break-up of prior land-masses, but before the establishment of Beringia and Wallacia. Time of the fewest continental connections	A, B to early C
20–5	Miocene area. Establishment of migratory routes between Asia and North America (AKA Beringia), Australasia and Southeast Asia (AKA Wallacia)	C
5–2.5	Pliocene epoch. Africa and Europe collide	D
2.5–0	End of Beringia: when North America separates from Asia. Central America is formed, joining North and South America	E, F

Ancestral host reconstruction

We performed ancestral state reconstructions (ASRs) of host associations in the supermatrix and supertree phylogenies. Parsimony and maximum likelihood methods were used to reconstruct the ancestral host for 10 nodes including the Sclerodermatineae, Core Sclerodermatineae, *Astraeus*, Diplocystidiaceae (Astraeus, Diplocystis and Tremellogaster, sensu Kreisel, 1974), *Boletinellus*, *Calostoma*, *Gyroporus*, *Pisolithus*, *Phlebopus*, and *Scleroderma*. States assigned to Sclerodermatineae taxa for all ASR analyses as well as a detailed description of coding strategies are given in Notes S1.

Two coding criteria were created to address ambiguity in host determinations. Under the liberal criterion, analyses used all host association data for ASR character coding assuming that fungal species were correctly identified. Analyses under the stringent criterion used only stringent definitions of host association for character coding. Under this criterion, the taxonomic identities of fungi were corroborated through DNA sequences or were justified using other evidence (e.g. geographic distribution limited to species).

We used MacClade v. 4.07 (Maddison & Maddison, 2005) for parsimony ASR analyses of ancestral hosts. Parsimony ASR was performed using the phylogenetic supertree because it contained the most taxonomically inclusive data set. Liberal and stringent coding for host character states used binary and multi-state methods. Binary state coding defined host states as either angiosperms or gymnosperms. Multi-state coding defined host states by host family association. The host families for analyses include: Betulaceae, Cistaceae, Dipterocarpaceae, Ericaceae, Fagaceae, Fabaceae (Mimosoideae and 'Caesalpinioideae'), Myrtaceae, Nyctaginaceae, Pinaceae, Oleaceae, Polygonaceae, Salicaceae, and Sapindaceae (Notes S2). Both binary and multi-state coding allowed taxa to be polymorphic in their host associations.

Maximum likelihood ASR analysis was implemented in BayesTraits (Pagel *et al.*, 2004). Using the 112-taxon supermatrix data set, we coded host character states using similar binary and multi-state methods, described in the preceding paragraph, under the stringent criterion. Under the liberal criterion, host association for a single species was assigned to each member of that genus. This was to maximize usage of host

association data from the literature for species that were not represented in this smaller data set. Multi-state coding required a more inclusive taxonomic ranking than that of the host family in order to reduce the number of host states for maximum likelihood analyses. These character states are gymnosperms (Pinaceae and Gnetaceae), Caryophyllales (Nyctaginaceae and Polygonaceae), eurosids I (Betulaceae, Fagaceae, Fabaceae, Nothofagaceae and Salicaceae), eurosids II (Cistaceae, Dipterocarpaceae and Sapindaceae), Myrtales (Myrtaceae) and asterids (Ericaceae and Oleaceae). The classifications for higher plants are provided by the Angiosperm Phylogeny Website (Stevens, 2001 onwards; http://www.mobot.org/MOBOT/research/APweb/). Maximum likelihood binary and multi-state analyses were performed on 100 posterior sampled phylogenies produced from Bayesian analyses. The average probabilities from all 100 analyses were calculated for each character state occurring at a node.

Results and Discussion

Phylogenetic analyses

Fig. 1 is one of 100 RAxML phylograms from the multi-gene supermatrix analyses with 106 Sclerodermatineae OTUs comprising *c.* 64 species. This phylogenetic tree is congruent with the Bayesian consensus tree, though slightly more resolved. The Sclerodermatineae (1.0 posterior probability) was resolved with multiple clades strongly supported by both maximum likelihood bootstrap (MLB) percentages and posterior probabilities (PPs). *Phlebopus* and *Boletinellus* were resolved as sister taxa with 98% MLB and 1.0 PP. These genera were resolved sister to the Core Sclerodermatineae (100% MLB; 1.0 PP), which represents six gasteromycete genera, *Astraeus* (97% MLB; 1.0 PP), *Calostoma* (100% MLB; 1.0 PP), *Scleroderma* (90% MLB; 1.0 PP), *Pisolithus* (99% MLB; 1.0 PP), *Diplocystis* and *Tremellogaster*, along with the hymenomycete genus *Gyroporus* (100% MLB; 1.0 PP). Both *Tremellogaster* and *Diplocystis* are monotypic genera that are consistently resolved as closely related to *Astraeus* (Louzan *et al.*, 2007), and in our study receive 82% MLB support (Fig. 1). This clade corresponds to the Diplocystidiaceae described by Kreisel (1974).

The 24 *Gyroporus* isolates in the data set are represented by approximately eight names, but these are distributed over 16 nonmonophyletic terminals (Fig. 1). Only 10 species are described for the genus, suggesting that there is much cryptic diversity (Kirk *et al.*, 2008). This result is similar for *Scleroderma*, where multiple OTUs of species *Scleroderma citrinum* and *Scleroderma areolatum* do not form monophyletic species groups. While this could be a result of misidentification, cryptic species have been reported in other Sclerodermatineae genera, including *Pisolithus* and *Astraeus* (Martín *et al.*, 2002; Phosri *et al.*, 2007). The results for Bayesian analysis of ITS data sets in *Astraeus*, *Calostoma* and *Phlebopus* are presented and discussed in Notes S3.

The MRP supertree (Fig. 2) resolves each of the strongly supported monophyletic genera from the Bayesian supermatrix tree and resolves additional species within *Astraeus*, *Pisolithus*, and *Calostoma* (Notes S3). Several taxonomic relationships within *Astraeus* and *Pisolithus* were not resolved in the strict consensus of all 8480 most parsimonious trees. This may be a consequence of combining numerous taxa from the ITS trees with the relatively small clades in the supermatrix tree. By contrast, *Calostoma* has greater taxonomic representation in the supermatrix tree (Fig. 1), and most of the relationships are resolved in the strict consensus supertree (Fig. 2).

Divergence times in the Sclerodermatineae

The XML files for the BEAST analysis parts 1 and 2 are provided in Notes S9 and S10, respectively. The consensus tree result for part 1 and tMRCAs for parts 1 and 2 of this analysis are presented in Notes S4. The resulting tMRCAs for clades representing marasmioid fungi and the Suillineae fall within the expected ages given the fossil record. The median age for the Sclerodermatineae was 82.47 Ma with a 95% HPD of 54.74–115.43 Ma, while the Core Sclerodermatineae was *c.* 58.24 Ma with an HPD of 34.48–84.95 Ma. These values were used to establish tMRCAs for the Sclerodermatineae and Core Sclerodermatineae in part 2 of the analysis.

The results of divergence dating in the Sclerodermatineae (part 2) are displayed in Fig. 3 with the tMRCAs and HPDs summarized in Table 3 and Notes S4. The median age estimated for the Sclerodermatineae (80.46 Ma; HPD 59.81–109.64 Ma) is only a couple of million years younger than estimates from part 1 (82.47 Ma; HPD 54.74–115.43 Ma), whereas the Core Sclerodermatineae (66.02 Ma; HPD 49.27–90.28 Ma) is nearly 8 million years older than the estimates of part 1 (58.24 Ma; 34.48–84.95 Ma) (Notes S4). This places the crown ages for the Sclerodermatineae and Core Sclerodermatineae in the late Cretaceous. Diversification of the Core Sclerodermatineae took place in the early Cenozoic era (Fig. 3). The crown ages for the groups within the Core Sclerodermatineae are younger than the Core Sclerodermatineae crown age by a minimum of 23 Ma. The tMRCA for *Calostoma* (42.73 Ma; HPD 28.78–61.69) makes it the oldest Sclerodermatineae group, followed by *Scleroderma* (38.37 Ma; HPD 26.26–53.71 Ma), Diplocystidiaceae (38.22 Ma; HPD 21.76–57.15 Ma), Bolletinellaceae (36 Ma; HPD 18.7–54.48 Ma), *Gyroporus* (34.58 Ma; HPD 22.66–48.9 Ma), *Pisolithus* (28.90 Ma; HPD 16.75–43.02 Ma), and *Astraeus* (15.62 Ma; HPD 7.63–24.13 Ma), which represents the youngest member in the Sclerodermatineae (Table 3). The results of divergence dating analysis suggest that Sclerodermatineae ancestors are young enough to have initially associated with each of its current host families. As a result it is possible for ancestral Sclerodermatineae to have associated with the ancestors of current Sclerodermatineae ectomycorrhizal associates.

Ancestral range reconstruction in the Sclerodermatineae

The Lagrange scripts used to evaluate the ancestral geographic ranges for the Sclerodermatineae, representing both range constraints and both dispersal rate models, are provided in

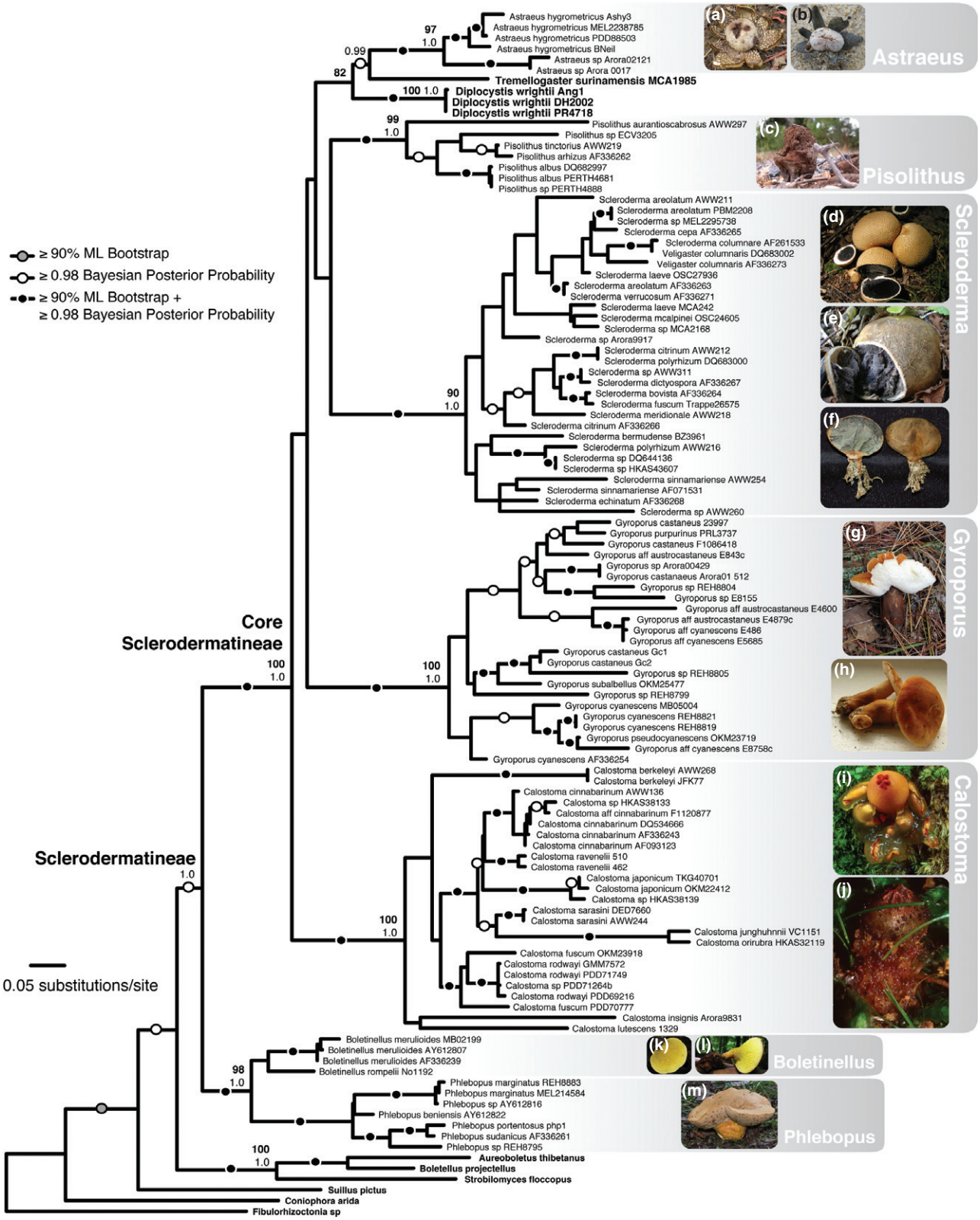

Fig. 1 One of 100 RAxML trees from the Sclerodermatineae supermatrix composed of 112 25S, 41 5.8S, 37 RPB1, 40 RPB2 and 31 ef1α sequences. Numbers adjacent to branches represent maximum likelihood bootstrap percentages (in bold) and Bayesian posterior probabilities. Images: (a) *Astraeus pteridis*; (b) *Astraeus* sp.; (c) *Pisolithus tinctorius*; (d) *Scleroderma citrinum*; (e, f) *Scleroderma* sp.; (g, h) *Gyroporus castaneus*; (i) *Calostoma cinnabarinum*; (j) *Calostoma rodwayi*; (k, l) *Boletinellus merulioides*; (m) *Phelbopus marginatus*. (Photo credits: (a), (b), (f) A. Wilson; (c) Darvin DeShazer; (d) Herbert Baker; (e) Tim Sage; (g) Eric Smith; (h) Michael Waisberg; (i) Mike Wood; (j) Christopher Dunk; (k) Eva Skific; (l) Dan Molter; (m) Ian Dodd).

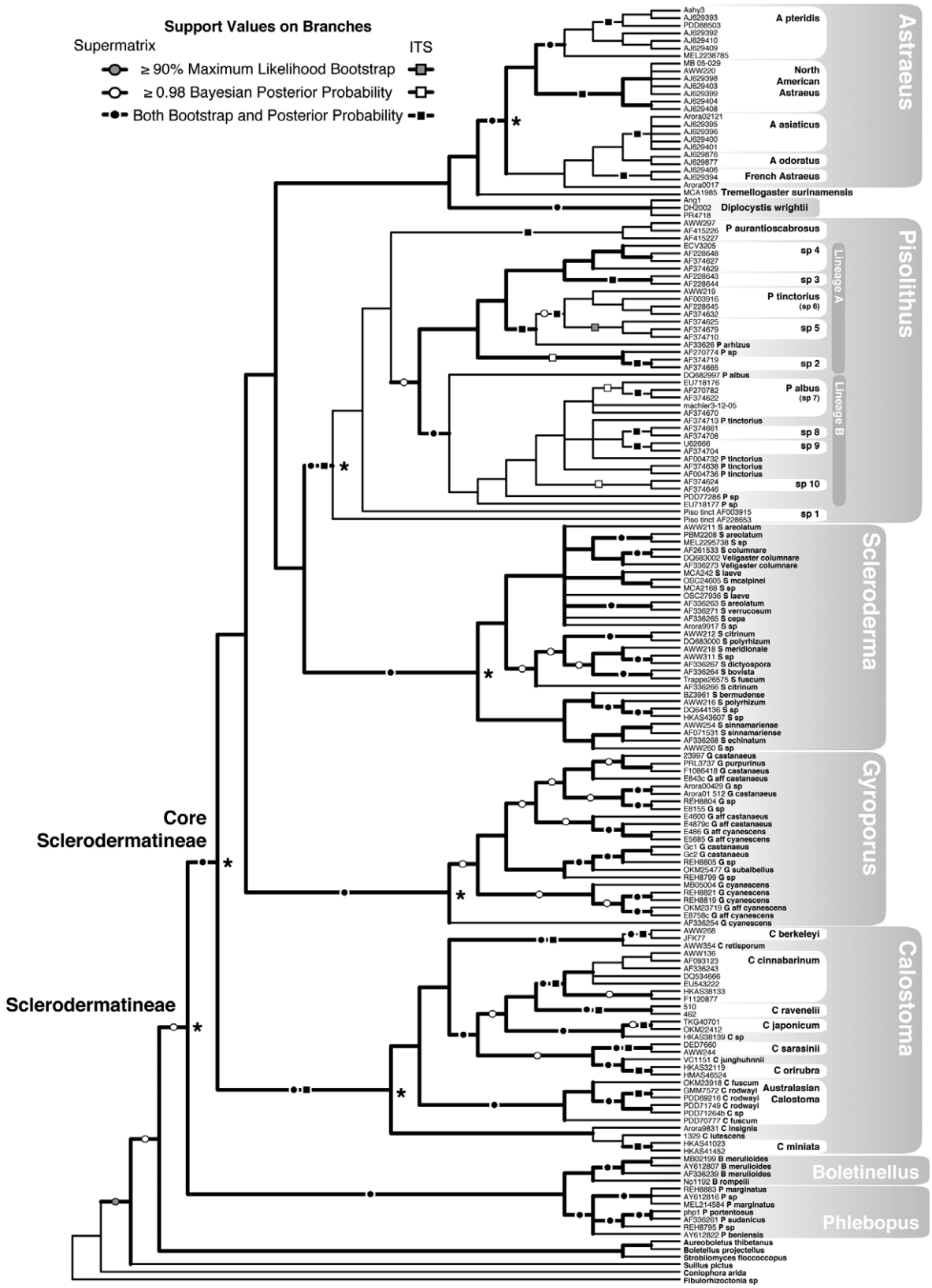

Fig. 2 Sclerodermatineae matrix representation using parsimony (MRP) supertree consisting of 168 taxa and assembled from phylogentic analyses of supermatrix and internal transcribed spacer (ITS) molecular data sets. One of 8480 most-parsimonious trees is shown. Bold branches indicate relationships that were resolved in the strict consensus analysis. Support for branches is indicated as circles or squares and was obtained from the supermatrix analyses or individual phylogenetic analyses of ITS data sets, respectively. Asterisks represent nodes constrained in MRP analyses.

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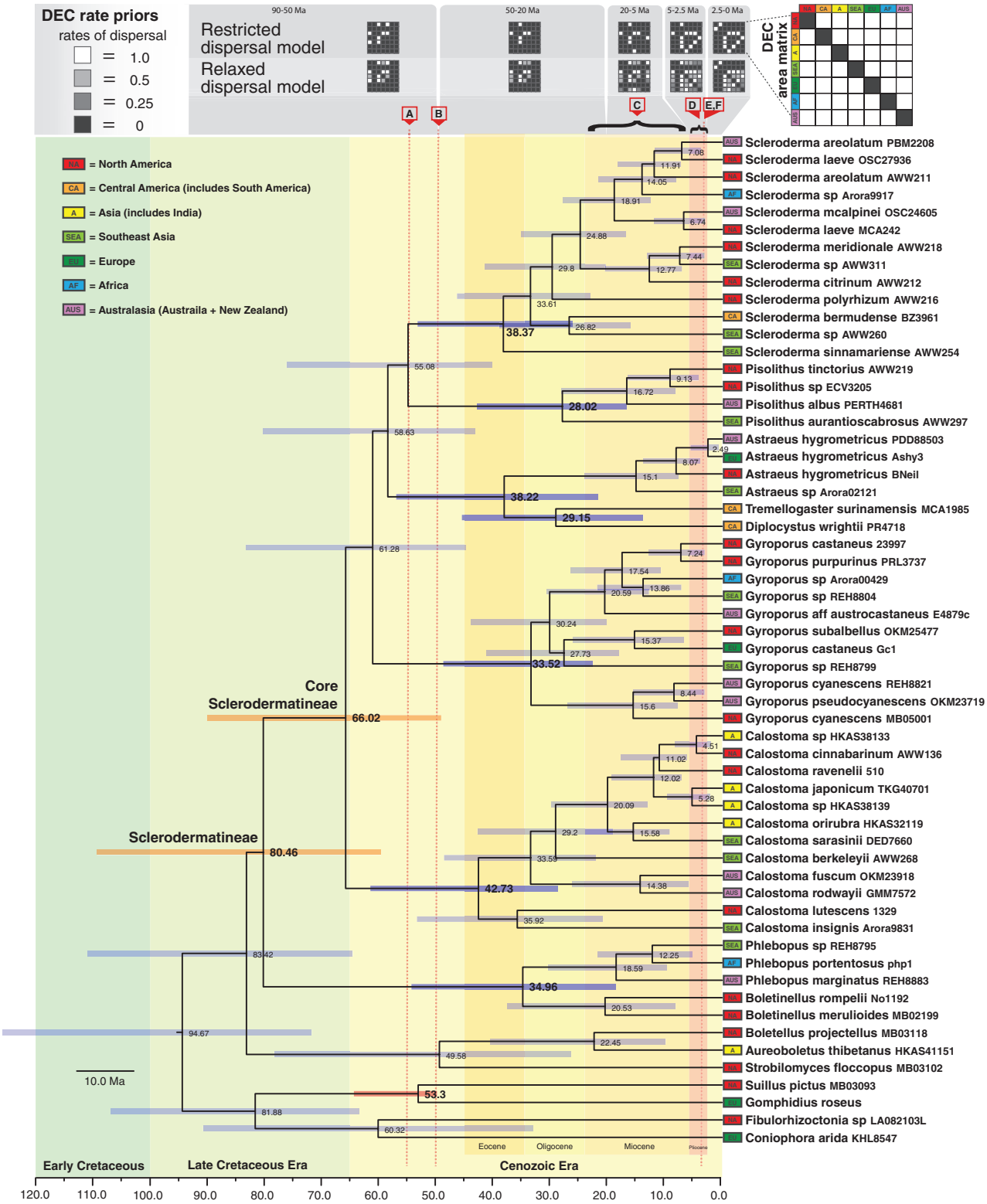

Fig. 3 Divergence time estimations in the Sclerodermatineae. Numbers next to nodes represent mean ages in millions of years ago (Ma) while bars represent 95% highest posterior densities (HPDs). Dark blue and orange bars identify time to most recent common ancestor (tMRCA) nodes. Orange and red bars identify calibrated nodes. Dispersal-extinction-cladogenesis (DEC) analysis priors for models and their corresponding area-dispersal rate matrices applied during defined age ranges are illustrated at the top of the figure. Geographical areas for DEC analysis are indicated at the terminal branches for Sclerodermatineae taxa. Letters A–F at the top of the figure identify geological events defined in Table 1.

Notes S11. The results of DEC analysis, reconstructing the ancestral ranges for major Sclerodermatineae groups, are displayed in Fig. 4(b) and summarized in Table 3. DEC range probabilities are provided in Notes S6.

The majority of reconstructions across the Sclerodermatineae favored an ancestral range centering on Asia, Southeast Asia, and North America. The result that was given the greatest probability, under the range constraint of ≤ 3 and both restricted and relaxed dispersal models, was a North and Central American ancestral range for the Sclerodermatineae and Core Sclerodermatineae (Fig. 4b). However, under this constraint, the probability for this ancestral range becomes progressively weaker in other groups of Sclerodermatineae, with the exception of the Diplocystidiaceae. This exception is probably attributable to the origins of *Diplocystis* and *Tremellogaster*, which are located in Central America, more specifically in the Caribbean and northern South America, respectively.

Under a range constraint of ≤ 2, almost all Sclerodermatineae groups have the greatest probability of an ancestral range in Asia and/or Southeast Asia under both dispersal models (Fig. 4b). Almost no species of Sclerodermatineae represented in this study are found in more than two areas defined in our study. However, collections of *Calostoma cinnabarinum* used in this study were also found in North America, Central America, and Asia (China) (Notes S1). Under this constraint Asia is the ancestral area with the greatest probability for Sclerodermatineae, Core Sclerodermatineae, *Astraeus* and Boletinellaceae. Southeast Asia is the ancestral area with the greatest probability for *Calostoma*, *Gyroporus* and the Diplocystidiaceae. The results for this last group conflict with the results found using the ≤ 3 range constraint. This could be because *Astraeus* taxa are lending more weight to an Asian origin for the Diplocystidiaceae when ancestral ranges are constrained to ≤ 2 areas. The ancestral ranges for *Pisolithus* and *Scleroderma* are nearly equivocal between Asia and Southeast Asia, giving a slightly higher probability to the latter area. Ancestral ranges with the greatest probabilities are summarized in Table 3.

Extant host associations in *Calostoma*

We confirmed the ectomycorrhizal status of *Calostoma sarasini* (FJ807559, FJ807561 and FJ807562) and *Calostoma retisporum* (FJ807564 and FJ807565) by matching fungal ITS sequences between ectomycorrhizal root tips and fruiting bodies. The ectomycorrhizal root tips of *C. sarasini* even have the gelatinous cuticle observed in *C. cinnabarinum* (Wilson *et al.*, 2007). BLAST searches using rbcL sequences (FJ807566, FJ807567 and FJ807568) from *C. sarasini* ectomycorrhizas as queries identified *Lithocarpus* sp. as the host/partner in the top three hits (AB125015, AB125013 and AB125014). This is the first report of a *Calostoma* species with *Lithocarpus*, and supports the *Calostoma*–Fagaceae relationship described by Wilson *et al.* (2007).

The BLAST search with rbcL sequences from *C. retisporum* ectomycorrhizas (FJ807569 and FJ807570) revealed the top three hits of *Tristaniopsis* sp. (AM235660), *Eugenia uniflora* (AM235654), and *Myrtus communis* (AF294254) as potential hosts. The identification of an ectomycorrhizal partner from the Myrtaceae is a first for *Calostoma*. However, *Calostoma fuscum* has been observed growing predominantly in *Eucalyptus* forests in Australia (C. Dunk, pers. comm.), suggesting that this association with the Myrtaceae extends to other *Calostoma* taxa. *Calostoma* in the southern hemisphere is also associated with *Nothofagus* in Australia and New Zealand. Although direct molecular evidence of this association has yet to be obtained, gelatinous ectomycorrhizal root tips similar in morphology to those of *C. cinnabarinum* have been observed growing on *Nothofagus* (C. Dunk, pers. comm.).

Extant host associations in other Sclerodermatineae

Sclerodermatineae taxa and their host associations are reported in Notes S2 along with literature references and the classification of host determination method. Forty-one studies describe hosts for 36 Sclerodermatineae species (Table 2), which are reported to form ectomycorrhizal symbioses with as many as 68 species in 15 plant families. Most gymnosperm references indicate associations with the Pinaceae, but Ingleby (1999) identifies *Scleroderma sinnamariense* as ectomycorrhizal with *Gnetum* using morphological identification of root mantle hyphae.

Only 37% of the host association reports can be considered 'stringent' (Table 2) based on our assessment of host association methods. These represent a little more than half of the plant families (n = 8) associated with the Sclerodermatineae. The literature describes more host associations with the angiosperms (n = 73) relative to the gymnosperms (n = 22). However, stringent methods were used to determine host associations in a little more than half of the gymnosperms (n = 12) compared with about a third of the angiosperms (n = 23). The greatest diversity of associations is with the angiosperms, where 13 families form ectomycorrhizal relationships with up to 52 Sclerodermatineae taxa. The host associations in *Pisolithus* and *Scleroderma* have been the most frequently studied, with 46 and 32 citations, respectively, while no more than six references were found for any of the remaining Sclerodermatineae genera.

The age and extant distribution for 12 of the 15 host families identified above are given in Fig. 4(a) and (c), respectively. Citations and references for the ages of Sclerodermatineae host families are provided in Notes S5. The Nyctaginaceae, Polygonaceae, and Cistaceae were not included because of a lack of information pertaining to their age, distribution and data describing ectomycorrhizal associations with the Sclerodermatineae (Notes S2).

Ancestral host reconstruction

ASRs are reported in Fig. 4(d). The MacClade file for parsimony ASR is presented in Notes S12. The results of reconstructions vary across analyses for the nodes. Binary and multi-state parsimony, and binary maximum likelihood ASRs gave the highest probability to a gymnosperm as the ancestral host at the root of the phylogeny (data not shown). For ancestral host states above the root, the reconstruction of either an angiosperm or a

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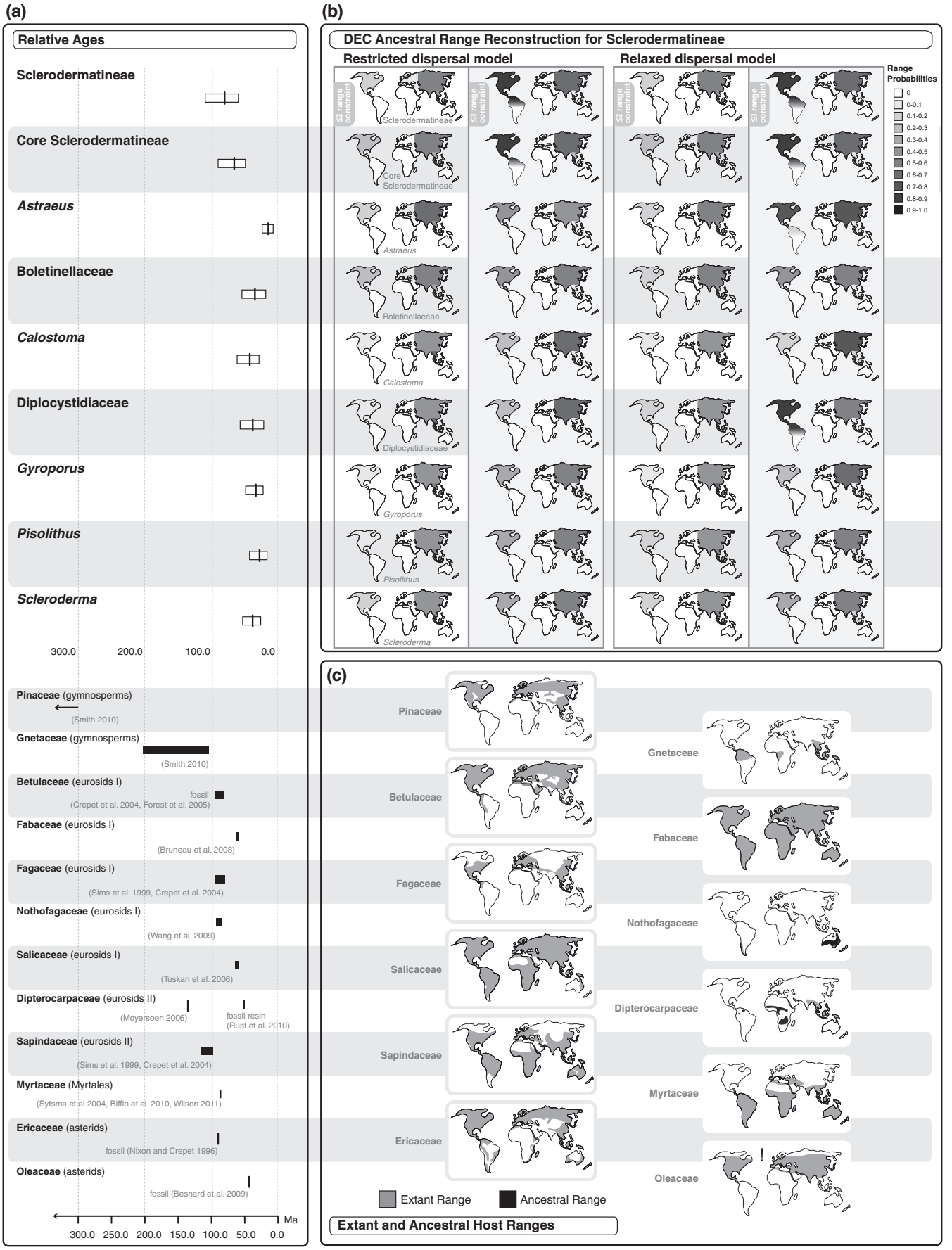

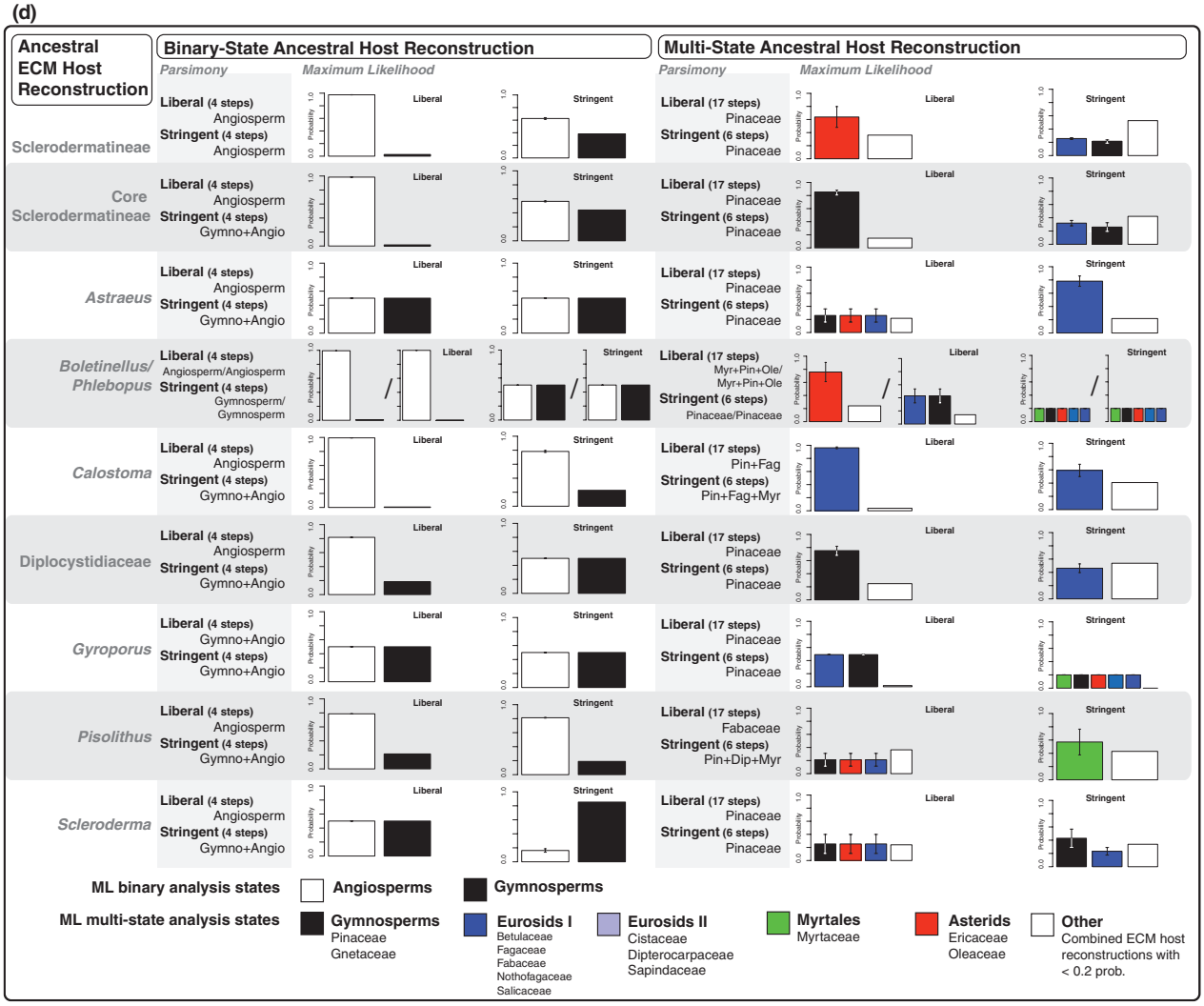

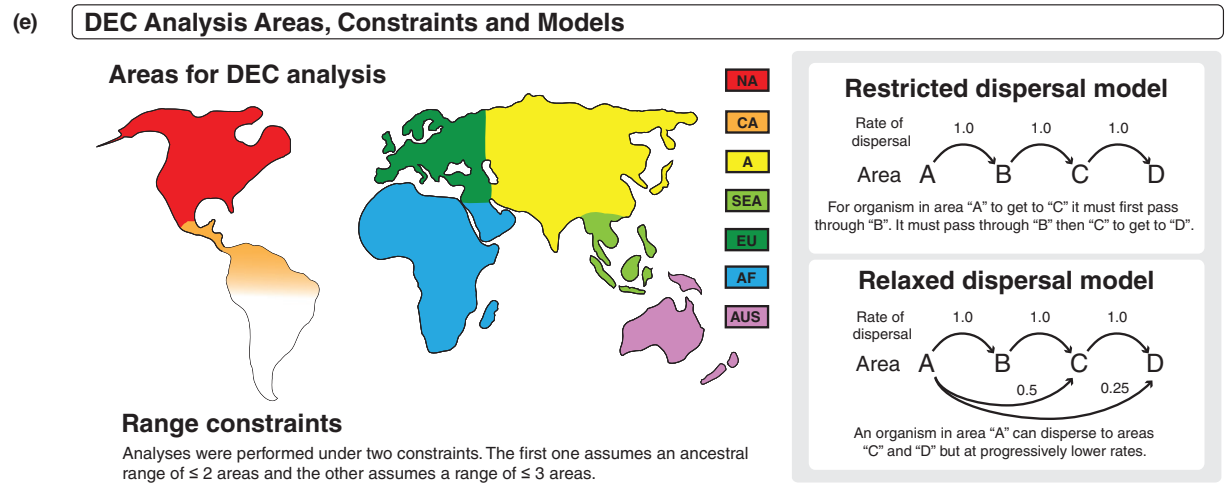

Fig. 4 (previous and current page) Relative ages, dispersal-extinction-cladogenesis (DEC) analysis ancestral range reconstruction, and ancestral ectomycorrhizal host reconstruction. (a) Relative ages of Sclerodermatineae groups and their putative ectomycorrhizal host families. Ages are expressed in millions of years ago (Ma). (b) DEC ancestral range results under restricted dispersal and relaxed dispersal models. Maps in the left column of each model represent constrained analyses limited to reconstructions of ≤ 2 areas, while the column on the right represent ≤ 3 areas constraint. Darker shades of gray indicate higher probabilities for the ancestral range. (c) Extant and ancestral ranges for putative ectomycorrhizal host families. Host ranges indicated with a '!' are reconstructions based on information gathered from online searches. (d) Ancestral ectomycorrhizal host reconstruction using binary-state and multi-state analyses, parsimony and maximum likelihood methods, under stringent and liberal coding criteria. Parsimony results indicate tree length (in steps) for ancestral host reconstructions, and the hosts reconstructed for the clade indicated. Maximum likelihood results are given as bars representing probabilities for different host groups. Bars indicate 95% highest posterior density (HPD). (e) Illustrated description of DEC parameters used in this analysis.

Table 2 Summary of Sclerodermatineae host association information described in the literature

	Total		
References	41		
Total Sclerodermatineae species	37		
Number of Sclerodermatineae associated host taxa described from literature	Total	Number of citations under defined host determination method: Stringent	Liberal
Plant host families	15	8	14
Total gymnosperm	2	2	1
Total angiosperm	13	7	13
Number of Sclerodermatineae ECM associations described from literature	Total	Number of citations under defined host determination method: Stringent	Liberal
Cited ECM associations	106	36 33.96%	70 66.04%
Astraeus	7	5	2
Boletinellus	1	0	1
Calostoma	3	3	0
Diplocystis	4	0	4
Gyroporus	2	0	2
Phlebopus	1	1	0
Pisolithus	49	11	38
Scleroderma	38	16	22
Tremellogaster	1	0	1
Gymnosperms	25	12	13
Angiosperms	80	23	57
Sclerodermatineae taxa associated with			
Gymnosperms	13	9	6
Pinaceae	12	9	5
Gnetaceae	1	0	1
Angiosperms	58	15	46
Betulaceae	4	1	3
Caesalpinioideae	4	1	3
Dipterocarpaceae	8	2	7
Ericaceae	4	4	0
Fagaceae	13	2	12
Mimosoideae	3	0	3
Myrtaceae	13	5	10
Nothofagaceae	1	0	1
Nyctaginaceae	1	0	1
Polygonaceae	1	0	1
Salicaceae	2	0	2
Sapindaceae	1	0	1
Cistaceae	2	0	1
Oleaceae	1	0	1

gymnosperm ancestral host for a particular node varied across analyses (e.g. *Phlebopus* under stringent and liberal binary analyses, or Core Sclerodermatineae under liberal binary and multi-state analyses). However, in the case of *Calostoma* and *Pisolithus*, reconstructions unambiguously favor an angiosperm as the ancestral host (Fig. 4d).

Reconstructions in the Sclerodermatineae and Core Sclerodermatineae across all multi-state analysis support ancestral hosts from the Pinaceae and eurosids I. These results largely suggest that an angiosperm ancestor is the most probable ancestral host; a result found in the study of other ectomycorrhizal groups (Hosaka *et al.*, 2008; Matheny *et al.*, 2009). However, the Pinaceae were resolved as the most probable ancestral host in parsimony multi-state ASR. This difference may be attributable to the coding sensitivity of parsimony when the overall weight of angiosperms from the binary-state analysis was reduced in the multi-state analysis as a result of it being fragmented into smaller constituents. The challenge of reconstructing ectomycorrhizal host associations is discussed in recent works by Ryberg *et al.* (2010)and Ryberg & Matheny (2011). The ambiguity between ASR results could also stem from sensitivities in interpreting ectomycorrhizal associations from the literature in coding (liberal vs stringent), different methods of data usage (binary vs multi-state), and/or different methods of analysis (parsimony vs maximum likelihood). The development of new stringent host association data could help resolve some of these ambiguities. Ultimately, our analyses suggest that the Pinaceae and rosid angiosperms played an important role as host to ancestral Sclerodermatineae and Core Sclerodermatineae.

The ancestral association with the asterids was produced in the multi-state, liberal coding analysis (Fig. 4d). This is potentially another example of coding sensitivity with the signal originating from *Fraxinus* (Oleaceae) in the Boletinellaceae. This association is dismissed as a possible ancestral host because the ectomyocorrhizal association between *Boletinellus* and *Fraxinus* is seen as dubious (Wang & Qiu, 2006; Tedersoo *et al.*, 2009). The ectomycorrhizal nature of *Phlebopus* is also considered dubious because, even though it has been shown to produce ectomycorrhizas *in vitro* (Sanmee *et al.*, 2010), it was not able to do so with *Fagara coco*, despite being associated *in silva* (Nouhra *et al.*, 2008). Ultimately, the Boletinellaceae association with plants is not disputed in this study, but the nature of this association needs further investigation.

A summary of age, range and host reconstruction information is presented in Table 3, with the last column identifying the probable ectomycorrhizal hosts based on a consideration of these data. Reconstructions of Core Sclerodermatineae lineages describe a number of host gains and losses that include new associations with the Dipterocarpaceae, Ericaceae, Myrtaceae, and Sapindales (Table 3). The present study used the parsimony ASR data set to evaluate host switching with MacClade (Notes S8). Overall, most host switching across binary/multi-state analysis and liberal/stringent coding occurs from Pinaceae hosts to angiosperm hosts. Within the angiosperms, host switching activity is largely centered around the Myrtaceae and the Fagaceae. These results are consistent with an ancestral association with the Pinaceae and numerous transitions to angiosperms, probably involving the Fagaceae and Myrtaceae.

Evolution of host associations in the Sclerodermatineae

Synthesizing the results from divergence dating, ancestral range, and ancestral host reconstruction analysis, it appears that the

Table 3 Summary of ancestral host reconstruction analysis in the Sclerodermatineae

Clade	Extant host families according to literature	Results from dating and reconstruction analyses			
		tMRCA (Ma)*	Ancestral range	Ancestral host	Likely ancestral hosts**
Sclerodermatineae	Betulaceae, Cistaceae, Dipterocarpaceae, Ericaceae, Fagaceae, Fabaceae, Gnetaceae, Myrtaceae, Nothofagaceae Nyctaginaceae, Pinaceae, Oleaceae, Polygonaceae, Salicaceae, Sapindaceae	(59.81–) 80.46 (–109.64)	Asia, North America, Asia + North America	*Gymnosperms*: Pinaceae; *Angiosperms*: Betulaceae, Fagaceae, Fabaceae, Salicaceae, Oleaceae	Pinaceae, Betulaceae, Fagaceae, Fabaceae, Salicaceae
Core Sclerodermatineae	Betulaceae, Cistaceae, Dipterocarpaceae, Ericaceae, Fagaceae, Fabaceae, Gnetaceae, Myrtaceae, Nothofagaceae, Nyctaginaceae, Pinaceae, Polygonaceae, Salicaceae, Sapindaceae	(49.27–) 66.02 (–90.28)	Asia, North America, Asia + North America	*Gymnosperms*: Pinaceae; *Angiosperms*: Betulaceae, Fagaceae, Fabaceae, Salicaceae	Pinaceae, Betulaceae, Fabaceae, Fagaceae, Salicaceae
Astraeus	Betulaceae, Ericaceae, Pinaceae	(7.63–) 15.1 (–24.13)	Asia, North America, Southeast Asia, Asia + Southeast Asia, Asia + North America	*Gymnosperms*: Pinaceae; *Angiosperms*: Betulaceae, Ericaceae, Fagaceae, Fabaceae, Salicaceae	Pinaceae, Betulaceae, Ericaceae, Fagaceae, Salicaceae
Boletinellaceae[1]	Dipterocarpaceae, Fabaceae, Myrtaceae, Pinaceae, Oleaceae	(18.70–) 34.96 (–54.48)	Asia, Southeast Asia, Asia + Southeast Asia	*Gymnosperms*: Pinaceae; *Angiosperms*: Betulaceae, Dipterocarpaceae, Ericaceae, Fagaceae, Fabaceae, Myrtaceae, Oleaceae, Salicaceae	Pinaceae, Dipterocarpaceae, Fabaceae, Myrtaceae, Oleaceae
Calostoma	Fagaceae, Myrtaceae	(28.78–) 42.73 (–61.69)	Southeast Asia, Asia, Asia + Southeast Asia	*Angiosperms*: Fagaceae, Myrtaceae	Fagaceae, Myrtaceae
Diplocystidiaceae	Betulaceae, Ericaceae, Fabaceae, Nyctaginaceae, Pinaceae, Polygonaceae	(21.76–) 38.22 (– 57.15)	Southeast Asia, North America, Central America, North + Central America,	*Gymnosperms*: Pinaceae; *Angiosperms*: Betulaceae, Fagaceae, Fabaceae, Salicaceae	Pinaceae, Betulaceae, Fagaceae, Salicaceae
Gyroporus	Fagaceae, Pinaceae	(22.66–) 33.52 (–48.90)	Southeast Asia, Asia, Asia + Southeast Asia	*Gymnosperms*: Pinaceae; *Angiosperms*: Fagaceae	Pinaceae, Fagaceae
Pisolithus	Betulaceae, Cistaceae, Dipterocarpaceae, Ericaceae, Fagaceae, Fabaceae, Myrtaceae, Pinaceae	(16.75–) 28.02 (–43.02)	Southeast Asia, Asia	*Gymnosperms*: Pinaceae; *Angiosperms*: Dipterocarpaceae, Ericaceae, Myrtaceae	Pinaceae, Dipterocarpaceae, Ericaceae, Myrtaceae
Scleroderma	Betulaceae, Dipterocarpaceae, Ericaceae, Fagaceae, Fabaceae, Gnetaceae, Myrtaceae, Pinaceae, Salicaceae, Sapindaceae	(26.26–) 38.37 (–53.41)	Asia + Southeast Asia, Southeast Asia, Asia, North America	*Gymnosperms*: Pinaceae; *Angiosperms*: Betulaceae, Ericaceae, Fagaceae, Fabaceae, Salicaceae	Pinaceae, Betulaceae, Fabaceae, Fagaceae, Salicaceae

*(2.5% HPD–) Median age (–97.5% HPD).

**Likely ancestral hosts are determined through a combined assessment of time to most recent common ancestor (tMRCA), ancestral range and ancestral host reconstructions.

[1]Although ectomycorrhizal relationships have been established in many Boletinellaceae species, it is likely that species of this group are only facultatively mycorrhizal.

HPD, highest posterior density.

Sclerodermatineae originated in Asia and North America during the late Cretaceous and diversified in the early Cenozoic, predominantly with the Pinaceae and rosids (Table 3). The modern consensus of angiosperm biogeography suggests that mixed mesophytic forests dominated the northern hemisphere during the early Cenozoic (Wolfe, 1975; Wen, 1999; Xiang & Soltis, 2001). These forests play an important role in the 'boreotropical hypothesis' which describes how disjunct distributions of extant neo- and paleotropical angiosperms were established via intercontinental land bridges (Wolfe, 1975; Tiffney, 1985). These forests were later fragmented during the Oligocene and Miocene as a result of the formation of intercontinental glaciers, disrupting dispersal routes between New World and Old World populations (Zachos *et al.*, 2001). The Fagaceae is an example of a boreotropical group, and an important Sclerodermatineae host, whose disjunct North American and Asian distribution is the result of vicariance in the Northern Hemisphere (Manos & Stanford, 2001).

The results of this study suggest that Sclerodermatineae ancestors dispersed with their ectomycorrhizal hosts in the early Cenozoic mesophytic forests (Wolfe, 1975, 1978; Gentry, 1988; White *et al.*, 1997; Buerki *et al.*, 2011). Around the time of the late Eocene/early Oligocene, the Core Sclerodermatineae began to diversify, but populations soon became fragmented as a result of climatic changes in the Oligocene and the break-up of their hosts' ranges. This study suggests that ectomycorrhizal associations with the Dipterocarpaceae, Ericaceae, Myrtaceae, Nothofagaceae, Sapindaceae, and potentially the Fabaceae were derived in later Sclerodermatineae lineages. This is based on the results of our ASR analyses (Fig. 4d; Table 3) and the different biogeographic histories for these hosts (Gentry, 1988; Sytsma *et al.*, 2004).

The phylogenetic assessment of the boreotropical hypothesis developed by Lavin & Luckow (1993) was applied to the Sclerodermatineae using area ASR parsimony analysis on the supertree data set (Notes S8, S13). Although the results are not entirely conclusive, some Sclerodermatineae clades do demonstrate a pantropical distribution that is consistent with the boreotropical hypothesis. This is interesting because it corroborates the suggestion of Matheny *et al.* (2009) that ectomycorrhizal fungi in the Northern Hemisphere dispersed according to hypotheses used to describe plant distributions. Future studies using expanded data sets should be able to demonstrate the importance of host associations to the dispersal of Northern Hemisphere ectomycorrhizal fungi.

Distributions of Sclerodermatineae outside of their ancestral host range can be explained by long-distance dispersal events, which requires host switching if dispersing to exotic habitats. The Sclerodermatineae have been shown to disperse long distances in a study of *Pisolithus* by Moyersoen *et al.* (2003). The New Zealand and Australian disjunct observed in *Pisolithus* species is also observed in other groups of fungi (Hosaka *et al.*, 2006; Moncalvo & Buchanan, 2008) and plants (Pole, 1994; Knapp *et al.*, 2005). Each of these studies rules out the possibility of ancient Gondwanan vicariance because of the age of the organismal groups involved. A long-distance dispersal capacity is demonstrated in other Sclerodermatineae as *Calostoma* also has this Australasian distribution. In addition, long-distance dispersal between North America and China appears to be possible in *C. cinnabarinum* according to the results of our ITS analysis (Notes S3). Vacariance probably has a role in the divergence of New World from Asian Sclerodermatineae. However, a more detailed sampling of Sclerodermatineae populations is needed to identify any significant effects of isolation in these fungi.

A generalist ectomycorrhizal habit probably facilitated the wide distribution of many Sclerodermatineae groups. This study supports Martín *et al.*'s (2002) suggestion that the ancestral *Pisolithus* was an ectomycorrhizal generalist. They also demonstrated the association between a broad geographic distribution and ectomycorrhizal promiscuity through *Pisolithus* associations with *Afzelia* (Fabaceae) in Africa (*Pisolithus* sp. 1), *Acacia* (Fabaceae) and *Eucalyptus* (Myrtaceae) in Australia (*Pislolithus albus*), *Cistus* in Spain (*Pislolithus* sp. 3), and *Pinus* (Pinaceae) and *Quercus* (Fagaceae) in Europe and North America (*Pislolithus* sp. 4 and *Pislolithus tinctorius*). The genus *Scleroderma* shares many of the same ectomycorrhizal associations as other broadly distributed Sclerodermatineae (Table 3), as demonstrated in recent studies describing *Scleroderma* species outside its ancestral range (Sanon *et al.*, 2009; Nouhra *et al.*, 2012). The current ASR results for *Gyroporus* are limited to the Pinaceae and Fagaceae (Table 3). This is probably a result of a paucity of information regarding the ectomycorrhizal host association for the genus (Table 2). Collections of *Gyroporus* from Australasia and Africa (Fig. 3) suggest an ability to form broader ectomycorrhizal associations that have yet to be defined. However, *Calostoma*'s host associations are similarly limited in ASR results and species have not been observed outside the distribution of hosts in the Fagaceae, Myrtaceae and Nothofagaceae. Overall, this suggests that the ability to form diverse host associations enables ectomycorrhizal fungi to distribute broadly.

The Diplocystidiaceae have North and Central America as their most probable ancestral range under the relaxed dispersal model and three-area constraint (Fig. 4b). Two Diplocystidiaceae species are limited to the neotropics. *Tremellogaster surinamensis* is found only in northern South America under *Dicymbe* ('Caesalpinioide') (Linder, 1930), while *Diplocystus wrightii* is found in the Caribbean with potential ectomycorrhizal hosts *Neea buxifolia* (Nyctaginaceae), *Coccoloba uviferea* (Polygonaceae), and *Pinus cubensis* (Louzan *et al.*, 2007). The ancestral range of *Astraeus* is similar to that of the Diplocystidiaceae (Fig. 4b) despite its current disjunct distribution (Fig. 3). It also shares several of the same ectomycorrhizal hosts as other Sclerodermatineae (Table 3). However, the biogeographic history of *Astraeus* requires further investigation as its age (*c.* 15 Ma) is too young for it to have evolved in the Eocene mesophytic forests. As a result the vicariance of the Oligocene could not have shaped the evolution of this group, as is suggested for other Sclerodermatineae.

The evolutionary history of the Sclerodermatineae is similar to that of other Boletales groups. The distributions of *Pulveroboletus* and *Tylopilus* are described by Halling (2001) as being 'relictually disjunct'. He suggests that *Pulveroboletus* is a relatively old genus found with Pinaceae and *Quercus* in North and Central America, with Myrtaceae and Casurinaceae in Australia, and with Fagaceae in Southeast Asian forests. This distribution is strikingly similar

to that of *Calostoma*, and both are also absent in Europe. In addition, Wolfe & Bougher (1993) describe *Tylopilus* as originating from Laurasia before migrating to Australasia over Pliestoscene land bridges and diversifying. The subgenus *Roseoscabra* is associated with Pinaceae, Fagaceae, Salicaceae, Myrtaceae, Mimosacaceae and Casurinaceae and distributed in the eastern USA, Costa Rica, Japan, China and northeastern Australia, but again is absent in Europe (Halling, 2001).

Conclusion

This study produced the most inclusive phylogenetic analyses for the Sclerodermatineae to date, to evaluate the evolution of ectomycorrhizal host associations using divergence dating, ancestral range and ancestral host reconstruction analysis. The results describe the Sclerodermatineae originating at the end of the Cretaceous with an ancestral range of North America, Asia and Southeast Asia, but many of the extant lineages did not diversify until the middle of the Cenozoic. During this time the Sclerodermatineae was ectomycorrhizal with as many as four host families (i.e. Pinaceae, Betulaceae, Fagaceae, and Salicaceae) that were associated with the Northern Hemisphere mesophytic forests of the early Cenozoic. Early Sclerodermatineae were distributed over a broad geographical area associated with its ancestral ectomycorrhizal hosts. The current distribution patterns seen in Sclerodermatineae are consistent with the boreotropical hypothesis proposed to describe the disjunct distribution of pantropical flora. Additional data and analysis may yield better insight into the importance of boreotropical flora in the evolution of ectomycorrhizal fungi. Extant distributions outside the Sclerodermatineae ancestral range are potentially attributable to a combination of long-distance dispersal capabilities and the ability to form diverse ectomycorrhizal associations.

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References

Agerer R. 1999. *Gyroporus cyanescens* (Bull.: Fr.) Quél. + *Pinus sylvestris* L. *Descriptions of Ectomycorrhizae* **4**: 43–47.

Binder M, Bresinsky A. 2002. Derivation of a polymorphic lineage of Gasteromycetes from boletoid ancestors. *Mycologia* **94**: 85–98.

Binder M, Hibbett DS. 2006. Molecular systematics and biological diversification of Boletales. *Mycologia* **98**: 971–983.

Binder M, Larsson K-H, Matheny BP, Hibbett DS. 2009. Amylocorticiales ord. nov. and Jaapiales ord. nov.: early-diverging clades of Agaricomycetidae dominated by corticioid forms. *Mycologia* **102**: 865–880.

Buerki S, Forest F, Alvarez N, Nylander JAA, Arrigo N, Sanmartín I. 2011. An evaluation of new parsimony-based versus parametric inference methods in biogeography: a case study using the globally distributed plant family Sapindaceae. *Journal of Biogeography* **38**: 531–550.

Creevey CJ, McInerney JO. 2005. Clann: investigating phylogenetic information through supertree analyses. *Bioinformatics* **21**: 390–392.

Danielson RM. 1984. Ectomycorrhizal associations in jack pine stands in north-eastern Alberta. *Canadian Journal of Botany* **62**: 932–939.

Drummond AJ, Ho S, Phillips M, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4**: e88.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**: 214.

Gentry AH. 1988. Changes in plant community diversity and floristic composition on environmental and geographical gradients. *Annals of the Missouri Botanical Garden* **75**: 1–34.

Halling RE. 2001. Ectomycorrhizae: co-evolution, significance, and biogeography. *Annals of the Missouri Botanical Garden* **88**: 5–13.

Hibbett DS, Grimaldi D, Donoghue MJ. 1997. Fossil mushrooms from Cretaceous and Miocene ambers and the evolution of homobasidiomycetes. *American Journal of Botany* **84**: 981–991.

Hibbett DS, Matheny BP. 2009. The relative ages of ectomycorrhizal mushrooms and their plant hosts estimated using Bayesian relaxed molecular clock analyses. *BMC Biology* **7**: 13.

Hosaka K, Bates ST, Beever RE, Castellano MA, Colgan W III, Domînguez LS, Nouhra ER, Geml J, Giachini AJ, Kenney SR *et al.* 2006. Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass Phallomycetidae and two new orders. *Mycologia* **98**: 949–959.

Hosaka K, Castellano MA, Spatafora JW. 2008. Biogeography of Hysterangiales (Phallomycetidae, Basidiomycota). *Mycological Research* **112**: 448–462.

Ingleby K. 1999. *Scleroderma sinnamarense* Mont+ *Gnetum africanum* Welw. *Descriptions of Ectomycorrhizae* **4**: 127–133.

Ji K-P, Cao Y, Ahang C-X, He M-X, Liu J, Wang W-B, Wang Y. 2011. Cultivation of *Phlebopus portentosus* in southern China. *Mycological Progress* **10**: 293–300.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. *Dictionary of the fungi*. Wallingford, UK: CAB International.

Knapp M, Stöckler K, Havell D, Delsuc F, Sebastiani F, Lockhart PJ. 2005. Relaxed molecular clock provides evidence for long-distance dispersal of *Nothofagus* (Southern Beech). *PLoS Biology* **3**: e14.

Kreisel H. 1974. Die Gattung *Diplocystis* und ihre stellung im system der Basidiomycetes. *Feddes Repertorium* **85**: 325–335.

Læssøe T, Jalink LM. 2004. *Chlorogaster dipterocarpi*: a new peristomate gasteroid taxon of the Sclerodermataceae. *Persoonia* **18**: 421–428.

Lavin M, Luckow M. 1993. Origins and relationships of tropical North America in the context of the boreotropics hypothesis. *American Journal of Botany* **80**: 1–14.

LePage BA. 2003. The evolution, biogeography and palaeoecology of the Pinaceae based on fossil and extant representatives. *Acta Horticulturae (ISIH)* **615**: 29–52.

LePage BA, Currah RS, Stockey RA, Rothwell GW. 1997. Fossil ectomycorrhizae from the Middle Eocene. *American Journal of Botany* **84**: 410–412.

Linder DH. 1930. Notes on *Tremellogaster surinamensis*. *Mycologia* **22**: 264–269.

Louzan R, Wilson AW, Binder M, Hibbett DS. 2007. Phylogenetic placement of *Diplocystis wrightii* in the Sclerodermatineae (Boletales) based on nuclear ribosomal large subunit DNA sequences. *Mycoscience* **48**: 66–69.

Maddison DR, Maddison WP. 2005. *MacClade 4*. Sunderland, MA, USA: Sinaur.

Manos PS, Stanford AM. 2001. The historical biogeography of Fagaceae: tracking the tertiary history of temperate and subtropical forests of the Northern Hemisphere. *International Journal of Plant Sciences* **162**(Suppl 6): S77–S93.

Martín F, Díez J, Dell B, Delaruelle C. 2002. Phylogeography of the ectomycorrhizal *Pisolithus* species as inferred from nuclear ribosomal DNA ITS sequences. *New Phytologist* **153**: 345–357.

Matheny BP, Aime MC, Bougher N, Buyck B, Desjardin DE, Horak E, Kropp BR, Lodge BJ, Soytong K, Trappe JM *et al.* 2009. Out of the Paleotropics? Historical biogeography and diversification of the cosmopolitan ectomycorrhizal mushroom family Inocybaceae. *Journal of Biogeography* **36**: 569–799.

Molina R. 1981. Ectomycorrhizal specificity in the genus *Alnus*. *Canadian Journal of Botany* **59**: 325–334.

Molina R, Trappe JM. 1982a. Lack of mycorrhizal specificity by the ericaceous hosts *Arbutus menziesii* and *Arctostaphylos uva-ursi*. *New Phytologist* **90**: 495–509.

Molina R, Trappe JM. 1982b. Patterns of ectomycorrhizal host specificity and potential among pacific northwest conifers and fungi. *Forest Science* **28**: 423–458.

Moncalvo J-M, Buchanan PK. 2008. Molecular evidence for long distance dispersal across the Southern Hemisphere in the *Ganoderma applanatum-australe* species complex (*Basidiomycota*). *Mycological Research* **112**: 425–436.

Moyersoen B, Beever RE, Martin F. 2003. Genetic diversity of *Pisolithus* in New Zealand indicates multiple long-distance dispersal from Australia. *New Phytologist* **160**: 569–579.

Nouhra E, Urcelay C, Becerra A, Dominguez L. 2008. Mycorrhizal status of *Phlebopus bruchii* (Boletaceae): does it form ectomycorrhizas with *Fagara coco* (Rutaceae)? *Symbiosis* **46**: 113–120.

Nouhra ER, Hernández Caffot ML, Pastor N, Crespo E. 2012. The species of *Scleroderma* from Argentina, including a new species from the *Nothofagus* forest. *Mycologia* **104**: 488–495.

Pagel M, Meade A, Barker D. 2004. Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology* **53**: 673–684.

Phosri C, Martín MP, Sihanonth P, Whalley AJS, Watling R. 2007. Molecular study of the genus *Astraeus*. *Mycological Research* **111**: 275–286.

Pole M. 1994. The New Zealand flora–entirely long-distance dispersal? *Journal of Biogeography* **21**: 625–635.

Raidl S, Scattolin L, Agerer R. 2006. *Gyroporus castaneus* (Bull.: Fr.) Quél. + *Quercus robur* L. *Descriptions of Ectomycorrhizae* **9**: 39–44.

Ree RH, Smith SE. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* **57**: 4–14.

Renner SS. 2005. Relaxed molecular clocks for dating historical plant dispersal events. *Trends in Plant Science* **10**: 550–558.

Ronquist F, Huelsenbeck J. 2003. MrBayes 3: Bayesian phylogenitic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Ryberg M, Larsson E, Jacobsson S. 2010. An evolutionary perspective on morphological and ecological characters in the mushroom family Inocybaceae (Agaricomycotina, Fungi). *Molecular Phylogenetics and Evolution* **55**: 431–442.

Ryberg M, Matheny PB. 2011. Dealing with incomplete taxon sampling and diversification of a large clade of mushroom-forming fungi. *Evolution* **65**: 1862–1878.

Sanmee R, Lumyong P, Dell B, Lumyong S. 2010. *In vitro* cultivation and fruit body formation of the black bolete, *Phlebopus portentosus*, a popular edible ectomycorrhizal fungus in Thailand. *Mycoscience* **51**: 15–22.

Sanon K, Bâ A, Delaruelle C, Duponnois R, Martín F. 2009. Morphological and molecular analyses in *Scleroderma* species associated with some Caesalpinioid legumes, Dipterocarpaceae and Phyllanthaceae trees in southern Burkina Faso. *Mycorrhiza* **19**: 571–584.

Sato H, Yumoto T, Murakami N. 2007. Criptic species and host sepcificity in the ectomycorrhizal genus *Strobiolomyces* (Strobilomycetaceae). *American Journal of Botany* **94**: 1630–1641.

Skrede I, Engh I, Binder M, Carlsen T, Kauserud H, Bendiksby M. 2011. Evolutionary history of Serpulaceae (Basidiomycota): molecular phylogeny, historical biogeography and evidence for a single transition of nutritional mode. *BMC Evolutionary Biology* **11**: 230.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.

Stevens PF 2001-onwards. *Angiosperm phylogeny website*. St Louis, MO, USA: Missouri Botanical Garden.

Swofford DL. 2003. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sunderland, MA, USA: Sinauer Associates.

Sytsma KJ, Litt A, Zjhra ML, Pires CJ, Nepokroeff M, Conti E, Walker J, Wilson P. 2004. Clades, clocks, and continents: historical and biogeographical analysis of Myrtaceae, Vochysiaceae, and relatives in the Southern Hemisphere. *International Journal of Plant Sciences* **165**: S85–S105.

Tedersoo L, May T, Smith ME. 2009. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* **20**: 217–263.

Tedersoo L, Suvi T, Beaver K, Kõljalg U. 2007. Ectomycorrhizal fungi of the Seychelles: diversity patterns and host shifts from the native *Vateriopsis seychellarum* (Dipterocarpaceae) and *Intsia bijuga* (Caesalpiniaceae) to the introduced *Eucalyptus robusta* (Myrtaceae), but not *Pinus caribea* (Pinaceae). *New Phytologist* **175**: 321–333.

Thoen D, Ducousso M. 1989. Mycorrhizal habit and sclerogenesis of *Phlebopus sudanicus* (Gyrodontaceae) in Senegal. *Agriculture, Ecosystems & Environment* **28**: 519–523.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**: 4876–4882.

Tiffney BH. 1985. Perspectives on the origin of the florisitic similarity between eastern Asia and eastern North America. *Journal of the Arnold Arboretum* **66**: 73–94.

Wang B, Qiu YL. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16**: 299–363.

Wen J. 1999. Evolution of Eastern Asian and Eastern North American disjunct distributions in flowering plants. *Annual Review of Ecology and Systematics* **30**: 421–455.

White JM, Ager TA, Adam DP, Leopold EB, Liu G, Jetté H, Schweger CE. 1997. An 18 million year record of vegetation and climate change in northwestern Canada and Alaska: tectonic and global climatic correlates. *Palaeogeography, Palaeoclimatology, Palaeoecology* **130**: 293–306.

Wilson AW, Binder M, Hibbett DS. 2011. Effects of fruiting body morphology on diversification rates in three independent clades of fungi estimated using binary state speciation and extinction analysis. *Evolution* **65**: 1305–1322.

Wilson AW, Hobbie EA, Hibbett DS. 2007. The ectomycorrhizal status of *Calostoma cinnabarinum* determined using isotopic, molecular, and morphological methods. *Canadian Journal of Botany* **85**: 385–393.

Wolfe CB, Bougher NL. 1993. Systematics, mycogeography, and evolutionary history of *Tylopilus* subg. *Roseoscarbra* in Australia elucidated by comparison with Asian and American Species. *Australian Systematic Botany* **6**: 187–213.

Wolfe JA. 1975. Some aspects of plant geography of the northern hemisphere during the late Cretacious and Tertiary. *Annals of the Missouri Botanical Garden* **62**: 264–279.

Wolfe JA. 1978. A paleobotanical interpretation of Tertiary climates in the Northern Hemisphere. *American Scientist* **66**: 694–703.

Xiang Q-Y, Soltis DE. 2001. Dispersal-vicariance analyses of intercontinental disjuncts: historical biogeographical implications for angiosperms in the Northern Hemisphere. *International Journal of Plant Sciences* **162**(Suppl 6): S29–S39.

Zachos J, Pagani M, Sloan L, Thomas E, Billups K. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292**: 686–693.

Supporting Information

Additional supporting information may be found in the online version of this article.

Notes S1 Sclerodermatineae taxa, GenBank IDs and character state assignments for ancestral host analysis. Detailed methodology for the assignment of host character states.

Notes S2 Sclerodermatineae host association references and methods of host association determination.

Notes S3 Bayesian ITS phylogenies for *Astraeus*, *Calostoma*, and *Pisolithus* and discussion of these results.

Notes S4 Molecular dating analysis part 1 results.

Notes S5 Sclerodermatineae host families, their ages, and references.

Notes S6 Dispersal-extinction-cladogenesis (DEC) analysis ancestral range probabilities for Sclerodermatineae groups.

Notes S7 Data set for molecular dating analysis part 1.

Notes S8 Sclerodermatineae data set for host switching analysis performed using parsimony.

Notes S9 .xml file for molecular dating analysis part 1.

Notes S10 .xml file for molecular dating analysis part 2.

Notes S11 Concatenated python scripts for dispersal-extinction-cladogenesis (DEC) analysis. Four scripts representing: (1) restricted model/two-area constraint, (2) restricted model/three-area constraint, (3) relaxed model/two-area constraint, and (4) relaxed model/three-area constraint.

Notes S12 NEXUS file for analyzing character state evolution and host switching in the Sclerodermatineae using parsimony.

Notes S13 Results of the boreotropical hypothesis test in the Sclerodermatineae using parsimony and the supertree data set.

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